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THE POSSIBILITY OF USING B. PRODIGIOSUM AS AN EXPERIMENTAL
BACTERIAL AEROSOL MODEL

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THE POSSIBILITY OF USING B. PRODIGIOSUM AS AN EXPERIMENTAL
BACTERIAL AEROSOL MODEL

[Following is the translation of an article by V. V. Vlodavets, Institute of General and Communal Hygiene imeni Sysina, AMN, USSR, appearing in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No. 11, 1964, pages 65--68. It was submitted on 11 Mar 1964. Translation performed by Sp/7 Charles T. Ostertag Jr.]

In the works of Soviet and foreign investigators quite often B. prodigiosum is used as the experimental model of a bacterial aerosol (Serratia marcescens of American authors or Chromobacterium prodigiosum in English literature). This microorganism forms a specific dark-red pigment and thus can be an unique indicator microorganism, easily differentiated from the microflora of the air. However, in the literature of recent years reports have started to appear more often concerning the weak stability of this microbe in the droplet phase of an aerosol. The death of this microorganism sets in particularly rapidly at low indices of relative air humidity (Thomas, 1955; Griffin et al., 1956; Vlodavets, 1957, 1962; Webb, 1959). Therefore, we undertook a special study of the stability of B. prodigiosum (strain No. 20-10) in an aerosol under the conditions of an experimental chamber with close temperature indices and wide fluctuations of relative air humidity.

The bacterial aerosol was created in an experimental chamber with a volume of 500 liters (type Ye aerosol chamber), in which an 0.3 ml suspension of a dry culture of B. prodigiosum in a physiological solution was sprayed. It contained 500 million microbial cells in 1 ml. The change in the concentration of viable cells in the air was determined over the period of a 4-hour test by the method of settling and with the help of a Rechmenskiy bacteria trap based on the number of colonies which grew on agar.

The viability of bacterial cells was also studied directly after the creation of the aerosol with the help of a method developed by us (Vlodavets, 1963). In the last case the recovery of air tests was done on membrane filters directly after the spraying of the bacterial

suspension. The membrane filters were placed for growing on the surface of a nutrient medium for five hours. We accepted as viable those cells which, following brief growing periods, formed microcolonies, since dead cells lost the capability for multiplication.

Inoculation by the method of settling, inoculation of fluid from the Rechmenskiy bacteria trap, and growing on membrane filters was carried out on glucose-starch agar, which ensured a sharp formation of the characteristic pigment by the bacteria.

The strain of B. prodigiosum used was characterized by the formation of the typical pigment, which in the various tests was preserved for the expanse of 10-15 passages. During subsequent passages, pigmentless forms and various pigment variants began to appear (rose, weakly rose, with a rose center), the number of which comprised 1-10%, as well as minute colonies. During subsequent passages the number of pigmentless colonies and pigment variants increased quite rapidly and reached 40-60% of the total population.

The results of the tests with the spraying of a suspension of B. prodigiosum showed that at low indices of relative humidity the extremely rapid death of bacteria in the air takes place, and with increased air humidity their survival rate increased, which was particularly noticeable at humidities exceeding 55-60% (table 1). Apparently the most favorable conditions were created at humidities above 70%. However, even under these conditions it was not possible to obtain a stable aerosol. The rapid lowering of the concentration of viable cells took place partially due to the settling of bacterial drops, but mainly as a result of their rapid dying off under the conditions of the air medium. At average and high indices of humidity, already in an hour following the dispersion of the bacterial suspension, in the air it was possible to determine viable bacteria in only 5-10% from their initial number. Subsequently their concentration decreased still more sharply.

Analogous regularities were noted in the recovery of air samples from the chamber with the Rechmenskiy bacteria trap and on membrane filters directly following spraying of the bacterial suspension. In the last case the number of non-multiplying (single) cells and the number of microcolonies on the membrane filters was determined (table 2).

Comparative data, obtained when determining viable bacteria directly after spraying and in the course of the next four hours, made it possible to make the conclusion that the dying off of B. prodigiosum at low indices of humidity took place mainly in the

first moments after the transfer of the bacteria from the liquid medium into the air. Also confirming this are the tests which we conducted with another strain of B. prodigiosum, and in which the air probes on membrane filters were recovered in the course of several seconds following the spraying of the bacterial suspension (Vlodavets, 1962).

In order to slow down the process of desiccation of the watery membrane of the bacterial drops, Clemmer et al. (1960) introduced 10% glycerin into the suspending liquid. We repeated these tests, however did not obtain a noticeable increase in the survival rate of B. prodigiosum at a low humidity. Apparently the dying off of microorganisms is a quite complex process, in which various factors play a role, and not only the rapid desiccation of the external watery membrane. In the opinion of Webb (1959), the death of microorganisms in the air takes place as a result of the loss of water which is bound with proteins and the subsequent disturbance in the structure of protein molecules.

We compared the results obtained with analogous investigations conducted with aerosols of Staphylococcus albus (Vlodavets, 1962, 1964). The latter, together with Staphylococcus aureus, are more stable under the conditions of the aerial medium of premises (Rochmenskiy, 1951; Klive and Vasilevskiy, 1952; Vershigora, 1958, 1960; Sinelnikova, 1961; Yaroshenko, 1962, and others). It was established by us that changes of air humidity in the chamber did not exert a significant influence on the concentration of viable staphylococci. During this the concentration of staphylococci was preserved somewhat longer at low indices of humidity and decreased at a high humidity (above 90%). These changes are apparently connected with the loss of viability by the staphylococci and may be explained by physiochemical processes taking place in the aerosols. During a study of an aerosol of Staphylococcus albus following spraying of the suspension, from 70 to 90% viable cells were detected regularly.

Thus, B. prodigiosum, in spite of the good indicator qualities, may be used for carrying out experimental investigations only at indices of relative humidity higher than 55% under the conditions of room temperature. Their death is minimal at a humidity higher than 70%. However, even under these conditions it is possible to conduct only short tests, since the stability of the bacterial aerosol is not great. At lower indices of humidity the utilization of B. prodigiosum as an experimental model is not advantageous due to the exceedingly rapid death of the bacteria in the air. It is also not expedient to use the stated species of microorganisms for a comparative evaluation of the effectiveness of instruments and for checking the effectiveness of air filtration (Noller and Spendlove, 1956). All these

difficulties in working with aerosols of B. prodigiosum are considerably increased as a result of the rapid increase in the population of the number of pigmentless forms and pigment variants.

Conclusions

1. The utilization of B. prodigiosum (strain No. 20-10) as an experimental model of an aerosol is not expedient due to the exceedingly rapid dying off of them at low indices of humidity, weak stability under the conditions of an air medium and the emergence of pigment variants. These microorganism may be used for the purposes of experimental aerobiology only with an air humidity greater than 60%.

2. There are specific advantages in using aerosols of Staphylococcus albus, which possesses a greater stability.

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Table 1

Effect of relative air humidity on the survival rate of *B. prodigiosum* in an aerosol following a ten minute exposure period.

Relative humidity (in %)	Temperature (in °)	Number of colonies grown in Petri dishes						
		Immediately after spraying	in various periods following completion of spraying					
			10 min	20 min	30 min	1 hr	2 hrs	4 hrs
20	20	30	0	0	0	0	0	
30	21.5	1 054	442	61	19	1	0	
41	19	588	312	185	165	24	0	
57	18	2 200	1 444	1 100	970	220	0	
68	19	2 196	1 540	877	311	103	0	
75	19	7 464	4 728	2 944	2 044	523	1	
95	18	10 432	7 444	5 600	4 488	1 020	4	

Table 2

Effect of air humidity on the survival rate of
B. prodigiosum directly following spraying

Relative humidity (in %)	Temperature (in °)	Number of bacterial cells	
		Live	Dead
13	18	0	100
20	20.5	0	100
30	23	2	98
44	18	14	86
56	19.5	53	42
60	18	76	24
78	19	82	18